GAMBIERTOXIN-INDUCED MODIFICATIONS OF THE MEMBRANE POTENTIAL OF MYELINATED NERVE FIBRES

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The effects of external application of 1.2–24nM of gambiertoxin (CTX-4B), extracted from the dinoflagellate *Gambierdiscus toxicus*, were studied on the membrane potential of myelinated nerve fibres isolated from the frog. At concentrations of 12 and 24nM, CTX-4B induced spontaneous action potential discharge at a frequency of 30–100Hz. In the presence of 24nM of CTX-4B, the amplitude and duration of these spontaneous action potentials were respectively decreased and increased compared to control action potentials. Toxin-induced spontaneous action potentials were suppressed by increasing the external calcium concentration or by lidocaine. It is concluded that the action of CTX-4B on membrane potential, in some respects, resembles that of moray-eel ciguatoxin previously reported (Benoit et al., 1986).

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Ciguatera toxins are responsible for the most common human food poisoning associated with the consumption of various tropical and subtropical fishes. The major source of these toxic compounds is the dinoflagellate, *Gambierdiscus toxicus* (Adachi & Fukuyo,1979).

Studies with ciguatoxin extracted from the moray-eel concluded that the toxin specifically interacts with Na channels of various preparations. In particular, in the frog node of Ranvier, the toxin induces spontaneous action potentials due to specific modifications of Na channels (Benoit et al.,1986).

In this work, we have investigated effects of the main toxic compound extracted from *G. toxicus*, gambiertoxin (CTX-4B), on the membrane potential of the frog myelinated nerve fibres.

MATERIALS AND METHODS

The experiments were carried out on single myelinated nerve fibres isolated from the sciatic nerve of the frog *Rana esculenta*. The membrane potential was recorded under current clamp conditions as previously described (Benoit et al.,1986). The Ringer's solution had the following composition (mM) : NaCl 111.5; KCl 2.5; $CaCl_2$ 1.8, HEPES 10; pH 7.4. The fibre ends were cut in a solution containing 120mM KCl. The temperature was 15–16°C.

Gambiertoxin (CTX-4B) was extracted from *Gambierdiscus toxicus* and purified. Samples were kept at –18°C and diluted immediately preceding experiments in Ringer's solution to give the final toxin concentrations indicated.

RESULTS

Effects of CTX-4B on Membrane Potential

CTX-4B at concentrations 1.2–6nM, applied for 12–22min, had no effect on the membrane potential, even when K currents were suppressed by replacing the solution bathing the cut fibre ends, i.e., the internal solution, with 110mM CsCl +10mM NaCl and by adding tetraethylammonium to make an external solution with a final concentration of 10mM. This was consistently observed on six different fibres, whether they were sensory or motor fibres.

In contrast, 2–8min after the addition of 12 or 24nM of CTX-4B to the Ringer's solution, spontaneous action potentials appeared at a frequency of 30–100Hz (Fig.1A). The toxin-induced spontaneous action potentials were often separated by silent periods and were less regular in amplitude and frequency than the spontaneous action potentials induced by moray-eel ciguatoxin (Benoit et al.,1986). Moreover, the resting membrane potential of fibres was apparently not modified by CTX-4B and maintained depolarizations to near –20mV were never observed, in contrast to the previously reported effects of moray-eel ciguatoxin (Benoit et al.,1986).

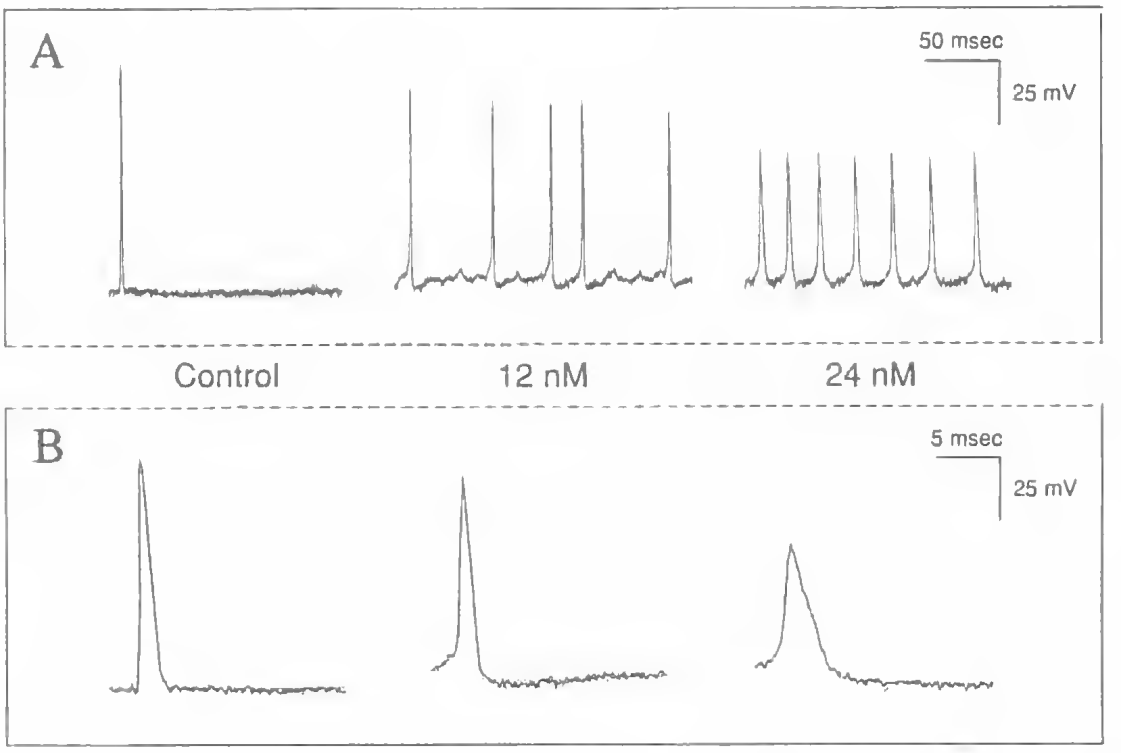

FIG.1. Control action potentials recorded in Ringer's solution evoked by 0.5msec depolarizing stimuli (left traces). Spontaneous action potentials recorded about 10min (middle traces) and about 12min (right traces) after bathing the nerve fibre in Ringer's solutions containing 12nM (middle traces) or 24nM (right traces) of CTX-4B. Note the difference in horizontal scales between (A) and (B).

In the presence of 12nM of CTX-4B, the amplitude of the spontaneous action potentials was only slightly reduced to 86±6% (n=3) of control on average and their duration, measured at the 50% level repolarization, was not significantly modified (mean 1.05±0.08%, n=3) compared to the control action potential elicited by a depolarizing stimulus (Fig.1B). This was not the case in the presence of 24nM of toxin, where the amplitude and duration of spontaneous action potentials were respectively reduced (mean 52±3%, n=4) and increased (mean 1.78±0.06%, n=4) compared to the control action potential (Fig.1B). Finally, it should be noted that the spontaneous action potentials induced by 12 or 24nM of CTX-4B (applied for 8–42min) were not suppressed after 18–36min wash out of toxin with control solution.

Effects of Tetraethylammonium on Spontaneous Action Potentials

Under control conditions, after the addition of tetraethylammonium to make the Ringer's solution with a final concentration of 10mM, the duration of elicited action potentials was 1.83±0.05 fold (n=3) greater than the duration of control action potential. Similar results were obtained when tetraethylammonium was added to a solution containing 12nM of CTX-4B, i.e., spontaneous action potentials were prolonged. In contrast, the subsequent addition of tetraethylammonium to the solution containing 24nM of CTX-4B or the addition of 24nM of toxin to the solution containing tetraethylammonium, did not noticeably further modify the duration of action potentials. As tetraethylammonium is well known to specifically suppress K current, these results strongly suggest that the nodal K current was not significantly affected by 12nM of CTX-4B, whereas it was reduced in the presence of 24nM of toxin.

Effects of Increasing External Calcium Concentration on Spontaneous Action Potentials

The effects of increasing the external con-

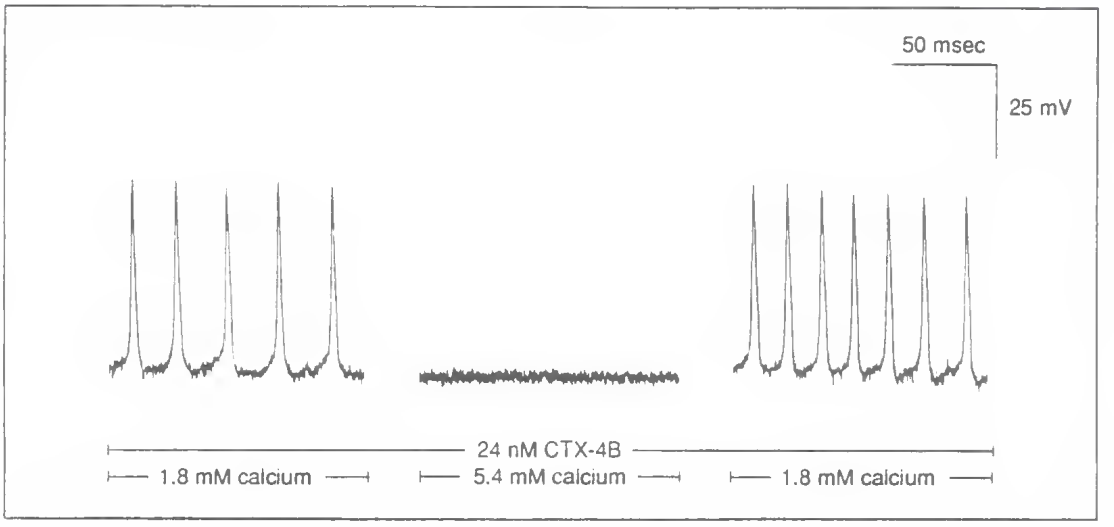

FIG. 2. Spontaneous action potentials recorded in a 24nM solution of CTX-4B made up in Ringer's solution containing successively 1.8mM (left trace), 5.4mM (middle trace) and 1.8mM of calcium (right trace).

centration of calcium were studied on spontaneous action potentials recorded in the presence of 12nM or 24nM of CTX-4B, or during wash out of CTX-4B with a Ringer's solution. Increasing the external calcium concentration from 1.8 to 5.4mM suppressed spontaneous action potentials in <30sec (Fig.2). In the presence of 5.4mM of calcium, an action potential could be elicited by a depolarizing stimulus. The effects of increasing external calcium concentration were reversed within 1–2min by superfusing the fibre with solutions containing 1.8mM of calcium (Fig.2).

Effects of Lidocaine on Spontaneous Action Potentials

Addition of 50μM of lidocaine to the Ringer's solution containing 12nM or 24nM of CTX-4B, inhibited spontaneous action potentials in less than 30sec (Fig.3). However, under such conditions, as previously described in the presence of 5.4mM of calcium, an action potential could be observed when the myelinated nerve fibre was stimulated by a depolarizing current. It should be noted that in the presence of CTX-4B, the frequency of appearance of spontaneous action potentials was 30–100Hz (Fig.1A) whereas the depolarization-induced action potentials were

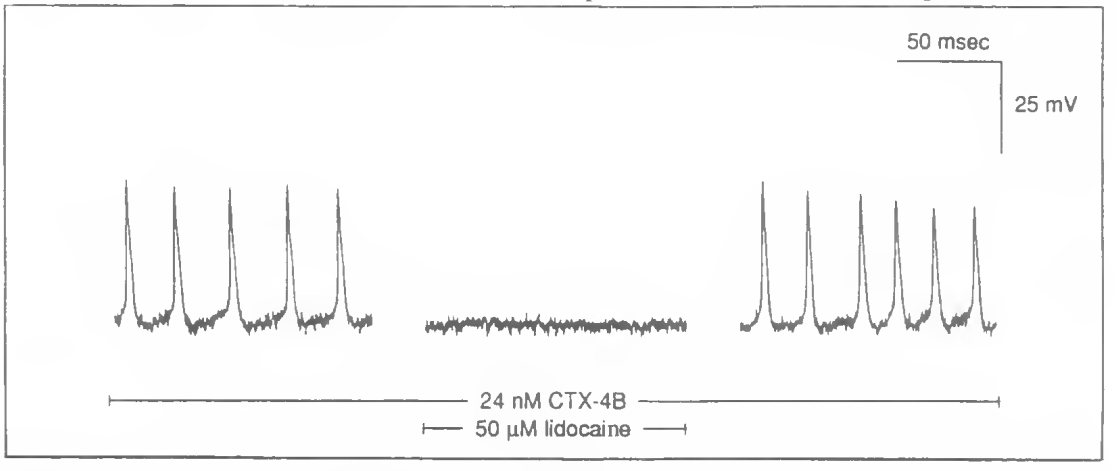

FIG. 3. Spontaneous action potentials recorded in a 24nM solution of CTX-4B made up in a Ringer's solution, in the absence (left trace), presence (middle trace) and after wash out (right trace) of 50μM of lidocaine.

usually elicited at a frequency of 1Hz. The inhibitary action of lidocaine has been shown to be more effective when the frequency of events is increased (Hille,1977). This may explain in part why lidocaine was more effective at blocking spontaneous action potentials than the elicited action potentials. The effects of lidocaine were reversed by a 2–3min wash with a Ringer's solution containing CTX-4B (Fig.3).

DISCUSSION

These results show that CTX-4B induces spontaneous action potentials in frog sciatic nerves which are suppressed by increasing the external concentration of calcium or by lidocaine.

Appearance of spontaneous action potentials was also observed in the presence of moray-eel ciguatoxin (Benoit et al.,1986). However, ciguatoxin was active at concentrations as low as 0.22nM, whereas in the present experiments concentration of at least 12nM of CTX-4B was needed to induce spontaneous action potentials. Thus, CTX-4B appears to be about 50 fold less effective than ciguatoxin on the membrane potential of myelinated nerve fibres. Finally, the effects of ciguatoxin were completely reversed upon washing out of toxin with a Ringer's solution, in contrast to those induced by CTX-4B.

It is concluded that, in some respects, the action of CTX-4B on membrane potential of myelinated nerve fibre resembles that of moray-eel ciguatoxin.

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